Effect of Seasonal Conditions and Soil Treatment on Bacteria and (Fungi) Molds in Soil

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
AND MECHANIC ARTS

P. E. Brown & W. V. Halversen

AGRONOMY SECTION

Soil Chemistry and Bacteriology

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THE EFFECT OF SEASONAL CONDITIONS AND SOIL TREATMENT ON BACTERIA AND MOLDS IN THE SOIL

By P. E. Brown and W. V. Halversen

The study of the microorganic population of the soil has revealed many interesting facts but none more significant nor of more far-reaching importance than the discovery that molds occur in soils and perform various functions which directly or indirectly exert considerable influence on soil fertility.

The occurrence and activities of bacteria in the soil has been the subject of extensive inquiry and much knowledge along this line has been accumulated. These organisms have been found to occur in large numbers in practically all soils and to play a prominent part in the reactions which must necessarily take place in soils in order that plant food shall be made available and crops properly nourished. In short, it has been definitely proven that bacterial activities bear a direct relation to soil fertility and to crop production and that permanent agriculture is very largely influenced by the presence and action of these microorganisms.

THE IMPORTANCE OF MOLDS IN SOIL

It appears from recent work, however, that bacteria are not the only living organisms which find a natural habitat in the soil and which affect crop growth because of their action on plant food constituents. Molds, protozoa, and algae have been found in many soils and new fields of study have been opened up in connection with each of these groups. More attention has been paid to molds and it appears probable now that they are of more importance than protozoa and algae and second only to the bacteria. Further study may possibly change this view, but the rank of molds among the soil organisms is really of secondary importance. It is more necessary now to study and attempt to solve some of the various fundamental problems involved in mold growth and action in the soil.

This work has only begun and while the investigations of the last few years have yielded much valuable information, they are far from complete. Years of investigation of bacteria in soil have been required in order to reach our present, still far from complete, knowledge of the relation of bacteria to soil fertility and there is no reason to hope for a short cut to

knowledge regarding molds. In fact, altho some information has been accumulated regarding bacteria which may apply to molds and some methods of study have been devised which may be employed to advantage, in general, molds differ so much from bacteria in many particulars that their study presents an entirely new problem involving certain new difficulties. It must be clearly understood, therefore, that much of our so-called knowledge of molds is, in reality, in need of confirmation and the evidence along many lines is insufficient to permit of definite conclusions.

A few facts, however, have been rather definitely proven and may be taken as the basis for further investigations. In the first place, it has been definitely established that molds commonly occur in soils and comprise an important group of soil organisms. Many species from a wide variety of soils have been isolated and described and an attempt has been made to show their common occurrence.

DIFFICULTIES ENCOUNTERED IN COUNTING MOLDS

In determining the actual number of molds present in soils, a difficulty has been encountered which has been deemed to vitiate seriously any accuracy which might pertain to the plate method. This difficulty arises because the plate counts show development not only of the active mycelia from the soil, but also of the spores. It is thus impossible to distinguish between the active and inactive mold forms in the soil. In fact, it has been claimed that molds probably occur in soil only in the form of spores and hence are unimportant. Waksman (16) suggested a method, however, by which it can be shown that molds live and produce mycelia in the soil. Conn (6) was unable to find mycelia present when he used his direct microscopic method of examination, but the writers (3), checking both methods, found active mycelia present in all the soils tested, even when using the smaller quantities of soil which Conn employed.

It has been deemed necessary to ascertain whether the molds are present in an active form in the soil, for it is claimed that the number of spores present means nothing inasmuch as the active forms are necessary if any influence on soil fertility is to result. This is, of course, very true, but the fact seems to be overlooked that the presence of mold spores in a soil not only shows the *previous* occurrence of active forms but, what is more important, it shows the future growth of mycelial forms. It is very easy under laboratory conditions to bring about the development of mycelia from spores and it seems reasonable to conclude that many spores in field soils will develop into active forms when the conditions for such development are provided.

Little is known of the specific conditions for individual organisms, but again it seems reasonable to conclude that if the conditions were once satisfactory in the field at some past time, they might be so again in the future. It has been claimed that the presence of mold spores in the soil does not necessarily mean the past occurrence of active forms, but may be due to dust infection. This suggestion does not seem reasonable, however, and it is not generally accepted.

It is believed that the number of molds in the soil is of great importance and while the active forms, of course, are most significant, the number of spores present may also give some idea of the future changes to be expected in the soil tested. While, therefore, the plate method does not distinguish between these two forms, it shows the total or potential mold content of the soil and may give results of considerable value.

The difficulties attendant upon the plate method are well known and it is unnecessary to discuss them here. In spite of these difficulties, however, the plate method is the only reasonable method yet devised for determining numbers of soil organisms. The direct microscopic method may possibly be so modified in the future that it will be utilizable, but at present Conn (8) himself admits its very grave limitations and points out the difficulties which attend its use. As employed to determine the number of spores and hyphae of molds in soil, the method is apparently quite as inaccurate as the plate method and indeed from Conn's own figures appears more so.

PURPOSE OF THESE INVESTIGATIONS

It was the purpose of the work reported here to study the relative numbers of bacteria and molds in variously treated soils, thruout an entire season and thus throw some light on the occurrence of molds in soil at different times of year and especially on the effect of temperature on mold growth. No attempt was made to distinguish between the active mold forms and the number of spores, and the plate method with all its limitations and uncertainties was used. Hence the results secured must not be interpreted too broadly nor the figures given considered entirely satisfactory. The relations established, however, may undoubtedly be considered rather definite and any influence of temperature shown, may be said to indicate quite distinctly the effect of seasonal conditions in the field on mold growth.

The effect of seasonal conditions on bacteria has been studied to some extent in the last few years, but the results secured have been somewhat conflicting. The results here reported should give some further information on the problem of bacteria in frozen soils and the relative effect of moisture and temperature on bacterial activities.

HISTORICAL

It is unnecessary to consider here the literature on the occurrence and action of molds in soils, as recent publications contain very complete bibliographies along this line (5, 18, 19), particularly the work of Waksman (19) which gives a thoro resume of the subject.

INVESTIGATIONS ON INFLUENCE OF TEMPERATURE ON MOLDS

Attention should be called, however, to the work on the influence of temperature on mold growth. Bartram (1) studied the effect of low temperature on certain fungi and bacteria and found that some fungi and bacteria are able to withstand extreme cold while others succumb to it. The temperatures used ranged down to —32° C. He found also that various organisms withstood exposure better in a dry condition than when food and moisture were present. Wolff (21) showed that certain fungi remained present and alive in Nebraska orchards thruout the winter. Severin (13) studied the microorganic population of soils from the far north (near the city of Obdorsk and on the Yamal peninsula) and found the soils rich in molds. Hagen (10) found that fungi live at as low a temperature as 6° to 8° C. Many developed at 12° to 15° C., but the optimum temperature was 20° to 25°.

Traaen (14) studied the temperature requirements of some of the soil fungi, measuring the growth of the organisms on agar plates under various temperatures. The organisms studied varied somewhat in temperature resistance, but the optimum was about 20° C. Coleman (5) studied the effect of temperature on five organisms using five temperatures: 6° to 8°; 15° to 17°; 22°, 30° and 38° C. He found that the fungi had a narrow temperature range. No activity as measured by ammonia accumulation was found at 6° to 8°. The minimum temperature seemed to be between 8° and 15° C. and the maximum was between 30° and 38° C. *Zygorhynchus Vuillemini* gave the greatest activity at 15° to 17°. *Rhizopus tritica* was most active at 22° to 25°. *Aspergillus niger* was most active at 30° and also at 38°, and it was the only organism remaining inactive at 15° to 17°. The other organisms studied besides those mentioned were a *Penicillium* and *Trichoderma Koningi.* Coleman concludes that the soil may be a determining factor in influencing the heat relations of soil fungi.

The early work on bacteria in frozen soils is discussed in the report of Brown and Smith (4) and need not be considered here. Since that report was made, Kossowitz (12) found smaller bacterial numbers in winter in some soils studied than in sum-

mer. He does not state whether or not the soil was frozen when tested. Weber (20) kept seven soils at temperatures of -10° to -20° C. for 14 days and found that low temperatures greatly increased numbers of bacteria. Given and Wills (9) found the lowest counts in the latter part of September when the soil was very cold, but not frozen. Fairly high counts were obtained when the soil was frozen, but *not* the largest of the year.

Harder (11) concludes that ordinary soil bacteria withstand cold to a marked degree, even to a temperature as low as 40° C. or more below zero. The increase in numbers seems to be due to mechanical transportation by moisture coming up from below during heavy frost, and where such transportation is not possible there is an actual retardation in growth as compared with that in unfrozen soils. This conclusion is directly contrary to that of Conn (7).

Waksman (17) found a high bacterial content in frozen soils, but not the largest thru the year. This may have been due to the fact that the soils under study were never frozen for a longer period than 8 to 10 days. The time of maximum bacterial development during the year varied with different soils. No two soils showed the maximum bacterial content at any one sampling. The lowest temperature studied was -2° C. Vanderleck (15) investigated frozen soils in Quebec and found that bacteria increased rapidly in January in all soils where there was raw material available for decomposition whether the soils were frozen or unfrozen. In March a moderate increase, equal to 2 to 4 times the original numbers, occurred. Severe frost checked bacterial development in frozen soils. A high water content counteracted frost action and a low water content assisted in depressing bacterial development. As soon as the soil thawed there occurred a decrease in bacteria. The second season's work confirmed the conclusions previously drawn and showed that severe frost checked bacterial development, the decrease being parallel to the depression in temperature. He found that slightly frozen conditions allowed of bacterial development, but his general conclusion was that in Canada no change took place in plant and crop remains during the winter as the temperature of the soil goes too low. This conclusion is in accord with the theory advanced by Brown and Smith (4) in their work. They believed that a temperature very much below zero would be necessary before the hygroscopic moisture would freeze and until that occurred a development of bacteria might be expected.

CONCLUSIONS FROM PREVIOUS WORK

From these experiments as a whole the conclusion seems warranted that bacteria may remain and be active in frozen soils, *provided* the temperature does not drop much below zero, and in some soils larger numbers may occur when the soil is frozen than after it has thawed. Apart from the studies of Brown and Smith (4) little is known as yet regarding the importance of these so-called "winter" bacteria from the fertility standpoint, or regarding their relation to the "summer" species. The quantity of plant food made available during the winter months is, therefore, also a matter of theory. Further work along this line is quite desirable. The results secured in the present work were incidental and for comparative purposes mainly, but they serve to show some interesting facts regarding the number of bacteria in frozen soils. They do not shed any light, however, on the action of "winter" and "summer" bacteria.

EXPERIMENTAL

Before taking up the main investigation, the purpose of which has already been mentioned, some preliminary studies were made of mold growth in the soil.

Waksman (16) in his tests of the occurrence of mold hyphae in the soil used portions of soil, 1 cm. in diameter. Conn (6) claimed that this amount of soil was too large. He found mycelia developing just as rapidly from conidia as from 10 mg. quantities of soil, altho when he used the same amount of soil as Waksman employed, active mold mycelia were apparently present. Conn also was unable to find mycelia present in soil by his direct microscopic method except where large quantities of organic matter were present.

PRELIMINARY TEST

The work of both these investigators was repeated, using the same quantities of soil which they employed. Agar plates were inoculated with soil and at the same time other plates were inoculated with spores and with portions of growing colonies from agar plates. After twelve hours incubation, fine mycelia were seen growing out from the particles of soil. At the end of seventeen hours these mycelia were very pronounced and at this time no growth had occurred from the spores. Even a greater growth occurred from the soil than from the portions of growing colonies. Smaller quantities of soil, than 10 mgs. were then employed and in every case mycelial development occurred more quickly than spores would germinate. The tests were not confined to soils particularly high in organic matter. It seems evident that molds occurred in an active form in all the soils tested.

A SECOND TEST

A second preliminary test was made to determine, if possible, the relative number of active molds and of spores in the soil. Soil was secured from one of the humus plots which is in continuous timothy meadow and contained 32.2 pct. moisture. Seven 100 g. portions of fresh soil were placed in 500 c. c. Erlenmeyer flasks containing 200 c. c. of sterile water. After shaking, the first infusion was plated on Cook's No. II medium. The other infusions were heated for varying lengths of time in boiling water and then plated. The actual temperatures of the infusions were ascertained by thermometers inserted thru the stoppers and extending into the infusions. The results secured are shown below.

Time Boiled	Bacteria	Molds	T. of Infusion
Check	5,068,000	109,000	
10 min.	381,000	27,000	88° C.
20 min.	55,000	....	96° C.
30 min.	....	....	96.5° C.
40 min.	....	....	96.5° C.
50 min.	....	....	96.5° C.
60 min.	....	....	96.5° C.

It appears that about 92 pct. of the bacteria were killed by heating for ten minutes. The hyphae of molds are no more resistant than bacteria and a similar effect might be expected. Only 75 pct. of the mould were killed, indicating that a considerable percentage of mold colonies on the plates came from spores. But as the 92 pct. of the bacteria killed in this heating serve as an index of the number of living bacteria in the soil, the 75 pct. of molds may be considered an index to the number of actaive molds in the soil. It is interesting to note that some of the bacteria withstood a greater period of boiling than the molds. No definite conclusions can be drawn from this test but it does serve to indicate that a rather large proportion of the colonies of molds developing on plates may represent active mycelial growth in the soil.

THE SOILS STUDIED

The soils studied in this work were taken from the humus plots of the station. The soil on these plots is classified as Carrington loam and the special treatments of the plots which have been followed since 1909 are as follows:

Plot 101—Continuous timothy meadow.
Plot 102—2.8 tons peat annually.
Plot 103—8 tons manure, once every four years (1909, 1913).
Plot 104—8 tons clover once every four years (1909, 1913).
Plot 106—2 tons timothy annually.
Plot 107—Check.

These plots are kept fallow and free from weeds except the timothy meadow plot, where the crop is cut and allowed to re-

main on the land. Samples were drawn from these plots with the usual precautions against contamination, the method described by Brown (2) being followed. When the ground was frozen a pick was used in place of a trowel. Samples were not taken for several days after a storm. Plates were prepared by the usual dilution method. Infusions were prepared by shaking 100 grams of soil with 200 c. c. of sterile water. One c. c. portions were transferred to 99 c. c. portions of sterile water (a); ten c. c. portions of (a) were transferred to 90 c. c. portions of water (b); ten c. c. of (b) into 90 c. c. portions (c) and ten c. c. (c) into 90 c. c. portions (d). One c. c. portions of the (c) and (d) dilutions were used to inoculate the Petri dishes. Two samples were drawn from each plot and triplicate plates were prepared from each sample.

THE MEDIA EMPLOYED

Three media were employed, Cook's No. II, Brown's albumen agar and Lipman and Brown's modified synthetic agar. The composition of these media is as follows:

	Cook's No. II	Albumen	Modified Synthetic
Distilled water	1000 c.c	1000 c.c.	1000 c.c.
Dextrose	20.0 gms.	10.0 gms.	10.0 gms.
Peptone	10.0 gms.	....	0.5 gm.
K_2HPO_4	0.25 gm.	0.5 gm.	0.5 gm.
$MgSO_4$	0.25 gm.	0.2 gm.	0.2 gm.
Egg Albumen	....	0.15 gm.	....
$Fe_2(SO_4)_3$	....	Trace	Trace
Agar	15.0 gms.	15.0 gms.	15.0 gms.

Cook's No. II is essentially a mold medium while the other two media allow the development of both bacteria and molds. The plates of Cook's No. II were incubated 4 days, while those of the other two media were incubated 12 days. In preparing the albumen agar the albumen was first mixed with a little water and to this a drop of NaOH was added, which caused the albumen to go entirely into solution. This was added to the medium after boiling and just before it was tubed.

Samples were drawn from the plots thruout the entire year at ten to twelve-day intervals, altho sometimes on account of storm the sampling was delayed several days. Twenty-six samplings in all were made.

The winter was cold and open and only during a small part of the time was the ground covered with snow. On December 11 the soil was frozen to a depth of about 1½ inches, and all samples from then to March 5 were taken from frozen soil.

Cook's No. II medium quite frequently gave a radically different count of bacteria than was obtained either on the albumen or the synthetic agar. This is due to the fact that this medium is especially adapted to the growth of molds, particular-

ly the mucors, and many of the molds grow so rapidly that they prevent the development of bacteria and other molds. Sometimes one-third of the plates were grown full of *Rhizopus nigricans* in two days. A longer incubation period would ordinarily be required for the molds than for the bacteria, especially if the former occurred only as spores. The count on Cook's No. II agar, therefore, after such a short incubation period, probably more nearly represents the actual numbers of living forms in the soil. At any rate the more active forms are represented, among them representatives of nearly all species. The mucors seem to be especially predominant.

CLIMATIC CONDITIONS

The climatic conditions thruout the year are shown in table I, which gives the air temperatures on each day of sampling, the rainfall for the month and the soil temperature in each plot. It will be seen that the soil temperature gradually dropped until January 16 when it reached —5.0 to —7.1° C. A rise in temperature occurred January 29, but this was followed by a drop on February 12 to -5.5° to -6.2°C and the temperature remained very low until March 5, after which it rose rapidly. The maximum was reached on August 6, 21.0° to 22.7° C. after which a drop occurred.

The rainfall decreased gradually from 1.81 inches in October to 0.26 in February. This was followed by an increase to 1.71

TABLE I—CLIMATIC CONDITIONS DURING PERIOD OF SAMPLING

Date of Sampling	Air Temp. on Day of Sampl'g Max.	Air Temp. on Day of Sampl'g Min.	Rain-fall for the Mo.	Soil Temperature Plot 101	Plot 102	Plot 103	Plot 104	Plot 106	Plot 107
1916	°C	°C	Inches	°C	°C	°C	°C	°C	°C
Oct. 17	1.7	-2.2	1.81	6.2	6.7	7.0	6.5	5.7	6.0
Oct. 28	17.8	10.5	1.81	9.0	9.0	9.0	9.7	9.7	9.7
Nov. 4	19.4	3.3	1.12	7.5	8.0	8.5	9.2	9.2	9.5
Dec 11	-0.5	-8.3	.94	1.5	1.2	1.0	0.9	1.4	0.5
Nov. 20	7.2	-4.0	1.12	2.7	2.5	2.5	2.5	1.0	1.0
Nov. 28	11.1	-4.4	1.12	3.2	3.2	3.0	3.2	3.0	3.0
Dec. 11	0.5	-8.3	.94	1.5	1.2	1.0	0.9	1.4	0.5
Dec. 18	-6.6	-18.9	.94	−1.5	−2.7	−3.0	−3.0	−4.0	−3.7
Dec. 27	−3.3	−15.5	.94	−1.2	2.0	−2.2	−2.5	−2.7	−3.0
1917									
Jan. 6	5.0	-10.5	.71	-2.5	-2.7	-2.7	-2.5	-2.0	-2.5
Jan. 16	-9.3	-20.5	.71	-5.0	-7.0	-6.7	-6.7	-7.1	-7.1
Jan. 29	0.0	-6.6	.71	-2.1	-2.2	-2.7	-2.0	-2.0	-2.1
Feb. 12	-3.9	-18.3	.26	-5.5	-6.0	-6.0	-6.0	-6.0	-6.2
Feb. 22	7.7	-16.7	.26	-3.5	-4.5	-5.0	-3.7	-4.7	-4.2
Mar. 5	0.0	-17.8	1.71	-4.0	-6.7	-6.0	-6.2	-5.0	-6.0
Mar. 24	18.3	-0.1	1.71	1.0	1.7	2.5	2.0	2.5	2.0
April 12	12.8	-0.1	5.0	4.5	5.0	6.0	6.0	5.0	5.0
May 3	6.7	0.5	4.01	7.5	7.0	7.7	7.2	6.7	6.5
May 29	21.7	8.3	4.01	12.5	16.0	15.7	16.0	16.0	15.0
June 19	26.1	10.0	8.59	16.5	18.2	19.0	18.7	19.2	19.0
June 30	37.2	18.3	8.59	18.7	22.0	22.3	22.3	22.0	22.2
July 18	29.4	12.8	1.93	20.7	25.0	25.5	25.0	27.0	26.0
Aug. 6	26.1	12.8	2.65	21.0	22.0	22.5	22.5	22.0	22.7
Sept. 3	25.0	15.6	1.83	18.2	21.0	21.0	20.7	21.0	21.0
Sept. 29	20.0	6.7	1.83	15.0	18.0	18.0	17.0	17.0	17.0
Nov. 2	13.3	-5.0	.58	14.5	13.2	13.0	13.2	13.0	13.0

inches in March, 5.0 inches in April, 4.01 inches in May, and 8.59 inches in June. After this date a decrease in precipitation occurred. The minimum for the year occurred in February and the maximum in June.

The moisture content of the soil in each plot was determined at each sampling and the tables and charts give these results. The results for Plot 101 may be taken as representative of all the plots, altho the differences are less marked in the other plots. From chart I, showing the results for plot 101, it appears that the moisture content varied very little until December 18, after which it increased until January 29, when a marked decrease was noted, altho the soil was frozen to a considerable depth. The high moisture content in January may be attributed to the fact that altho the precipitation was small both in December and January, the moisture was all held in the surface soil, being unable to penetrate the frozen soil. In February, however, the very light precipitation was insufficient to keep up the moisture content. From February to May 3, the moisture in the soil was fairly constant, but after the latter date a decrease occurred, due to accelerated evaporation and utilization by crops.

While the results secured with the other plots vary somewhat from these, in general the moisture curves agree very closely. They need not be considered further here, as they will be discussed in connection with the bacteria and mold data.

The moisture conditions on this series of plots while perhaps

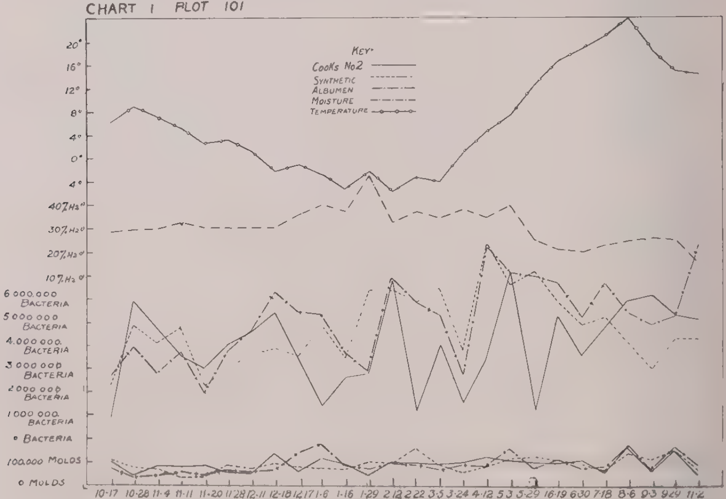

Chart I. Bacteria and molds in soil from continuous timothy plot

not exactly the same as would be found any other season, represent quite satisfactorily average field conditions and they may be considered of some value as a basis for general conclusions. The bacterial and mold growth during the particular season may, in other words, be taken as indicating what may occur under average seasonal conditions.

THE GROWTH OF MOLDS IN RELATION TO BACTERIA, MOISTURE AND TEMPERATURE

The results obtained in this work are given in tables II, III and IV, which show respectively the numbers of bacteria and molds growing on albumen agar, modified synthetic agar, and Cook's No. II agar thruout the year, twenty-six samplings in all being made. The moisture and temperature at each sampling are also shown in the tables. Charts are prepared for each plot and the results as plotted on these appear much more distinctly than in the tables. Curves for the numbers of bacteria and molds on each medium are given. The tables need not be discussed separately, therefore, but attention may be centered on the charts.

Considering the results on the continuous timothy plot as shown on chart I, it appears that the mold content was very little influenced by either moisture or temperature. Occasionally a high count was obtained as on January 6 on the albumen agar, but it is possible that some mold particularly adapted to growth on the albumen agar had fruited in the sample. In general, there seemed to be no effect from low temperatures in decreasing the number of molds. In fact, there was a gradual increase in numbers from the first sampling thru the time of lowest temperature and the highest count on all three media occurred when the highest temperature was recorded. At this time the lowest moisture content was found.

The general fluctuations in mold content did not seem to follow the variations in moisture content during any season of the year. This was true of all three media. Occasionally a higher or lower count on one medium seemed to agree with a higher or lower moisture content than at the previous date, but the results were so variable that any conclusion as to an effect of moisture would not be warranted. In fact, the variation in numbers of molds was not definite enough to permit of conclusions regarding the effect of any or all seasonal conditions. It might even be concluded that the number of molds fluctuates in soils without regard to moisture or temperatures—such fluctuations might be due to some other factor or to some condition connected with the obtaining of the counts or with the life cycle of the organisms.

TABLE II. ALBUMEN AGAR

Plot	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds
	October 17				October 28			
101	6.25	28.6	2,698,000	78,100	9.0	29.0	3,995,000	32,750
102	6.75	28.6	2,209,500	89,750	9.0	32.65	2,042,500	61,450
103	7.0	31.0	2,811,000	102,800	9.0	29.95	2,086,500	33,350
104	6.5	35.5	3,040,000	32,200	9.75	29.3	5,355,000	37,400
106	5.75	23.85	1,588,500	31,050	9.75	26.05	4,066,500	45,200
107	6.0	22.45	1,776,000	12,200	9.75	23.6	751,500	71,900
Ave.	6.37	28.33	2,354,000	57,700	9.37	28.52	3,046,000	47,000
	November 4				November 11			
101	7.5	29.45	2,809,000	42,700	5.5	32.05	3,725,000	60,700
102	8.0	31.4	2,940,000	60,750	5.0	32.35	2,247,500	51,650
103	8.5	31.85	4,600,000	119,650	5.25	31.0	3,128,500	52,250
104	9.25	29.3	5,380,000	59,450	5.0	29.2	4,572,500	85,350
106	9.25	24.3	3,800,000	48,400	5.75	26.7	4,152,500	58,250
107	9.5	22.6	2,647,500	72,350	5.25	25.25	1,029,750	57,600
Ave.	8.7	28.15	3,696,800	67,200	5.3	29.42	3,142,600	60,670
	November 20				November 28			
101	2.75	30.05	1,919,000	46,950	3.25	30.55	3,746,000	69,350
102	2.5	31.7	1,873,500	29,750	3.25	29.8	2,591,500	97,400
103	2.5	30.45	2,425,000	54,500	3.0	27.8	3,068,500	102,100
104	2.5	29.3	5,395,000	72,450	3.25	26.15	4,885,000	100,800
106	1.0	24.25	4,480,000	50,550	3.0	24.2	3,732,500	99,750
107	1.0	27.0	1,827,000	41,700	3.0	23.5	2,209,000	44,570
Ave.	2.4	28.8	2,986,600	49,310	3.12	27.0	3,374,100	85,666
	December 11				December 18			
101	1.5	30.35	4,675,000	60,100	–1.5	30.75	6,350,000	69,300
102	1.25	32.0	3,360,000	43,550	–2.75	31.25	3,637,500	51,200
103	1.0	30.3	2,723,000	34,350	–3	28.55	4,018,500	41,150
104	.87	25.65	3,067,500	32,400	–3	29.45	3,861,000	76,350
106	1.37	24.35	3,630,000	81,050	–4	26.5	3,385,000	101,900
107	.5	24.0	1,550,000	53,400	–3.75	22.9	1,900,000	45,400
Ave.	1.08	27.77	3,167,600	50,810	–3.0	28.23	3,858,700	64,220
	December 27				January 6			
101	–1.25	35.55	5,511,000	144,000	–2.5	40.05	5,372,500	186,900
102	–2.0	32.45	2,202,500	39,050	–2.75	44.3	2,256,000	67,400
103	–2.25	35.75	2,227,500	62,150	–2.75	41.65	3,110,000	102,600
104	–2.5	36.5	2,885,000	67,000	–2.5	38.25	5,395,000	63,600
106	–2.75	42.2	3,066,000	50,850	–2.0	26.8	2,651,000	79,850
107	–3.0	32.2	3,905,000	47,550	–2.5	25.1	1,794,000	49,800
Ave.	–2.3	35.92	3,299,500	68,430	–2.5	36.02	3,429,700	91,690
	January 16				January 29			
101	–5	37.2	3,665,000	90,350	–2.13	52.95	2,925,000	75,350
102	–7	41.05	4,597,500	74,350	–2.25	42.1	2,242,500	51,300
103	–6.75	39.0	3,900,000	73,150	–2.75	37.0	2,756,000	41,900
104	–6.75	36.95	6,775,000	68,600	–2.0	42.9	2,850,000	47,050
106	–7.13	36.0	4,272,500	72,900	–2.0	33.0	3,333,000	44,100
107	–7.13	32.8	2,011,000	64,850	–2.13	35.55	1,670,500	44,150
Ave.	–6.63	37.3	4,203,500	76,030	–2.5	40.6	2,629,500	60,640
	February 12				February 22			
101	–5.5	32.25	6,970,000	95,270	–3.5	37.15	5,805,000	93,750
102	–6	49.45	3,825,500	77,700	–4.5	46.05	4,242,500	58,400
103	–6	38.9	3,843,000	35,550	–5	35.05	4,015,000	57,050
104	–6	36.5	5,500,500	50,450	–3.75	41.35	5,455,000	99,900
106	–6	32.2	5,415,000	74,000	–4.75	27.25	4,670,000	71,200
107	–6.25	38.05	1,974,000	37,250	–4.25	34.55	1,548,500	106,000
Ave.	–6.0	37.06	4,688,000	61,700	–4.3	36.9	4,289,300	81,050
	March 5				March 24			
101	–4	34.4	5,415,000	72,100	1	38.2	2,790,000	96,500
102	–6.75	41.6	4,670,000	47,650	1.8	42.9	3,510,000	103,500
103	–6	28.85	3,990,000	49,550	2.5	37.1	3,806,000	90,000
104	–6.25	35.25	5,012,500	81,600	2.0	35.4	5,910,000	93,300
106	–5	29.8	4,552,500	64,970	2.5	31.5	5,285,000	113,000
107	–6.0	28.85	2,867,000	51,900	2.0	27.9	3,630,000	98,000
Ave.	–5.7	33.12	4,417,800	61,290	2	35.5	4,155,200	99,000

TABLE II. ALBUMEN AGAR (Continued)

Plot	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds
	April 12				May 3			
101	4.5	34.1	8,220,000	96,000	7.5	39.4	7,120,000	162,000
102	5	30.8	5,195,000	86,500	7.0	39.0	3,340,000	123,500
103	6	31.3	3,587,500	87,000	7.8	34.2	3,600,000	98,400
104	6	30	4,707,500	120,500	7.3	33.4	6,265,000	114,500
106	5	26	4,050,000	116,800	6.8	28.9	6,260,000	92,500
107	5	23.1	3,310,000	87,000	6.5	24.9	2,537,500	74,100
Ave.	5.2	29.2	4,845,000	98,900	7	33.3	4,853,700	110,800
	May 29				June 19			
101	12.5	24.2	7,060,000	82,000	16.5	20.9	6,803,000	176,300
102	16	27.5	5,625,000	187,000	18.3	27.5	5,050,000	76,500
103	15.8	26.1	4,785,000	58,500	19	25.8	7,000,000	50,300
104	16	25	6,165,000	57,500	18.8	25.6	9,315,000	56,700
106	16	23.1	6,010,500	44,600	19.3	23.5	6,810,000	106,000
107	15	20.7	3,132.000	62,500	19	21.1	2,870,000	67,300
Ave.	15	24.4	5,462,900	82,000	18.5	23.9	6,308,000	79,000
	June 30				July 18			
101	18.8	19.7	5,255,000	81,400	20.8	22.1	6,742,500	88,000
102	22	25.4	4,290.000	83,400	25	25.9	4,367,500	75,600
103	22.4	25.5	4,665,000	75,400	25.5	26.5	5,325,000	50,600
104	22 4	24.5	5,542.500	50,800	25	35.6	6,965,000	57,800
106	22	22.1	5,365 000	55,000	27	22.9	5,705,000	72,700
107	22.3	18.9	1,760,000	102,200	26	20.9	3,425,000	55.900
Ave.	21.6	22.6	4,479,600	74,700	24.9	23.9	5,421,700	66,800
	August 6				September 3			
101	21	24.4	5.450 000	181,500	18.3	25.4	4,985,000	78,700
102	22	24.9	3.937,500	99,000	21	26.6	3,416,000	87,000
103	22.5	24.1	3,490,000	85,500	21	25.2	4,230,000	57,500
104	22.5	23.5	6 305,000	97,500	20.8	24	5,281,000	77,000
106	22	21.8	4.252,500	101,500	21	21.1	5,045,000	123,500
107	22.8	19.3	2.262,000	63.000	21	18.5	1,545,000	54,700
Ave.	22	23	4,283,000	104,700	20.5	23.4	4,083,700	79,700
	September 29				November 2			
101	15	24.4	6,375,000	156,800	14.5	28.7	8,312,500	102,900
102	18	26.1	5.745.000	104,500	13.3	28.0	6,885,000	46,000
103	18	25.3	6.165.000	86,700	13	26.9	6.857,500	96,400
104	17	24.3	6.525,000	82,100	13.3	24.6	6,540,000	57,300
106	17	21 3	5,360.000	71,500	13	22.2	6,412,500	84,700
107	17	19.6	2,960,000	86,700	13	20.5	3,235,000	43,300
Ave.	16.8	23.5	5,522,000	98,000	13.3	25.1	6,373,800	71,800

TABLE III. MODIFIED SYNTHETIC AGAR

Plot	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds
	October 17				October 28			
101	6.25	28.6	2,266,500	103,200	9.0	29.6	4.855,000	78,250
102	6.75	28.6	1,290,000	135,250	9.0	32.65	4,030,000	44,250
103	7.0	31.0	1.968.500	61,400	9.0	29.95	1,409,000	59.850
104	6.5	35.5	2.786.000	70,200	9.75	29.3	5.447,500	24,500
106	5.75	23.85	1,964,000	116,600	9.75	26.05	4,242,500	40,350
107	6.0	22.45	1.262,000	142.000	9.75	23.6	2,419,000	40.600
Ave.	6.37	28.33	1,922.800	104,770	9.37	28.52	3,733,800	47,970
	November 4				November 11			
101	7 5	29.45	4.135.500	72,750	5.5	32.05	4.797,500	30,300
102	8.0	31.4	3.725,000	96,100	5.0	32.35	2.331,000	30,600
103	8.5	31.85	8.842.500	87,250	5.25	31.0	1,961,000	74,350
104	9.25	29.3	3.242 500	103,450	5.0	29.2	5,030,000	57,500
106	9 25	24.3	2,321,500	88,250	5.75	26.7	3,592,500	83,600
107	9.5	22.6	1,462,500	81,000	5.25	25.25	1,601,000	33,400
Ave.	8.7	28.15	3,964.900	88,130	5.3	29.42	3,218,800	51,620

TABLE III. MODIFIED SYNTHETIC AGAR (Continued)

Plot	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds
	November 20				November 28			
101	2.75	30.05	2,242,500	34,850	3.25	30.55	3,095,000	86,500
102	2.5	31.7	1,930,000	39,600	3.25	29.8	2,142,500	62,800
103	2.5	30.45	2,840,000	97,500	3.0	27.8	3,314,500	46,050
104	2.5	29.3	3,830,000	63,450	3.25	26.15	4,520,000	130,300
106	1.0	24.25	4,022,500	52,400	3.0	24.2	3,227,500	110,050
107	1.0	27.0	1,178,000	58,700	3.0	23.5	1,464,500	85,750
Ave.	2.4	28.8	2,673,800	57,750	3.12	27.0	2,960,700	86,910
	December 11				December 18			
101	1.5	30.35	3,645,000	65,000	–1.5	30.75	3,931,000	99,350
102	1.25	32.0	3,390,000	39,550	–2.75	31.25	3,557,500	74,250
103	1.0	30.3	3,106,000	34.000	–3	28.55	2,501,000	59,150
104	.87	25.65	3,302,500	112,500	–3	29.45	3,308,500	59,600
106	1.37	24.35	2,865,000	44,750	–4	26.5	3,195,000	70,600
107	.5	24.0	2.651,000	57,100	–3.75	22.9	2.630,000	52,550
Ave.	1.08	27.77	3,159,900	58,820	–3	28.23	3,187,200	69,250
	December 27				January 6			
101	–1.25	35.55	3,625,000	85.450	–2.5	40.05	4,916,500	78.200
102	–2	32.45	2,311,000	87,150	–2.75	44.3	2,445,000	56,050
103	–2.25	35.75	2,981,500	58,200	–2.75	41.65	2,201,500	54,300
104	–2.5	36.5	3,567,500	44,850	–2.5	38.25	5,303,500	77,350
106	–2.75	42.2	4.141,000	72,000	–2.0	26.8	3,482,500	49.600
107	–3.0	32.2	2,168,500	70,100	–2.5	25.1	1,227,500	50,150
Ave.	–2.3	35.92	3,132,400	69,620	–2.5	36.02	3,262,700	60,940

Plot	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds
	January 16				January 29			
101	–5	37.2	3,490,000	75.300	–2.13	52.95	6 492,500	100,550
102	7	41.05	1,165,000	107,050	–2.25	42.1	3,190,000	47,050
103	–6.75	39.0	2.583,000	68,250	–2.75	37.0	2 152,500	49,900
104	–6.75	36.95	5.255,000	90,500	–2.0	42.9	5 455 000	57,150
106	–7.13	36.0	3.762,500	89,650	–2.0	33.0	4,247,500	97.750
107	–7.13	32.8	1,577.500	55,900	–2.13	35.55	1,847,500	31,850
Ave.	6.63	37.3	2,968,800	81,110	–2.5	40.6	3 897,500	65,710
	February 12				February 22			
101	–5.5	32.25	6,492,500	100,550	–3.5	37 15	5 910 000	167,500
102	–6	49.45	4,585,000	75,350	–4.5	46.05	3,750,000	62 650
103	–6	38.9	3,867,000	67,750	–5	35.05	4,780.000	56.400
104	–6	36.5	5.476,000	63,800	–3.75	41.35	4 952,500	51,050
106	–6	32.2	3,777,500	74,000	–4.75	27.25	4,357,500	49.450
107	–6.25	38.05	1,882,000	50,600	–4.25	34.55	1,832.000	150 550
Ave.	6.0	37.06	4,346,700	72,010	–4.3	36.9	4,263.700	89.600
	March 5				March 24			
101	–4	34.4	6,535,000	90.250	1	38.2	3,780 000	63,600
102	–6.75	41.6	3.645,000	52.750	1 8	42.9	2 820 000	110,000
103	–6	28.85	3,817,500	34,900	2.5	37.1	4,577,500	72,800
104	6.25	35.25	4.141.500	67,550	2.0	35.7	5.470 000	71 800
106	–5	29.8	4.875,000	52.000	2.5	31.5	4.985 000	77.300
107	–6	28.85	2,575,000	43.000	2.0	27.9	5 315 000	66.500
Ave.	–5.7	33.12	4.264.800	56.740	2	35.5	4.491 300	77.000

Plot	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds
	April 12				May 3			
101	4.5	34.1	8,400,000	88.500	7.5	39.4	6.680.000	120 000
102	5	30.8	4,420,000	60,300	7.0	39.0	4,545 000	147,000
103	6	31.3	4.410,000	93.500	7.8	34.2	4 325 000	96,800
104	6	30	4.675,000	59.500	7.3	33.4	6,620 000	69,700
106	5	26	4,350.000	115.000	6.8	28.9	5,445.000	79 500
107	5	23.1	2.545.000	50.000	6.5	24.9	2,570.000	61.800
Ave.	5.2	29.2	4,800,000	77,800	7	33.3	5 030.800	95,800
	May 29				June 19			
101	12.5	24.2	7.295,000	131,500	16.5	20.9	5,935 000	116.300
102	16	27.5	4.900,000	80,500	18.3	27.5	4,815.000	38 200
103	15.8	26.1	4.250,000	63,400	19	25 8	8,655 000	45.400
104	16	25	6,165,000	86,400	18.8	25.6	7 335 000	56.500
106	16	23.1	6.465.000	81,500	19.3	23.5	8.165.000	93.200
107	15	20.7	2,703,000	79,500	19	21.1	2,742 500	64,500
Ave.	15	24.4	5.296.300	87.100	18.5	23.9	6,274.600	69.400

TABLE III. MODIFIED SYNTHETIC AGAR (Continued)

	June 30				July 18			
Plot	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds
101	18.8	19.7	4,932,500	95,800	20.8	22.1	5,230,000	72,000
102	22	25.4	4,895,000	82,000	25	25.9	3,770,000	58,000
103	22.4	25.5	3,880,000	41,400	25.5	26.5	3,765,000	45,600
104	22.4	24.5	3,552,500	77,400	25	35.6	5,375,000	66,600
106	22	22.1	3,850,000	56,600	27	22.9	5,447,500	81,200
107	22.3	18.9	2,254,000	60,800	26	20.9	1,548,500	64,400
Ave.	21.6	22.6	3,897,000	69,000	24.9	23.9	4,189,300	64,600
	August 6				September 3			
101	21	24.4	....	140,800	18.3	25.4	3,004,000	121,000
102	22	24.9	....	77,500	21	26.6	2,063,500	67,200
103	22.5	24.1	....	127,900	21	25.2	1,798,000	56,400
104	22.5	23.5	....	161,800	20.8	24	3,966,000	57,200
106	22	21.8	....	146,500	21	21.1	2,830,000	72,700
107	22.8	19.3	....	69,000	21	18.5	1,180,000	51,000
Ave.	22	23	....	120,600	20.5	23.4	2,473,600	70,900
	September 29				November 2			
101	15	24.4	4,395,000	153,500	14.5	28.7	4,380,000	79,100
102	18	26.1	4,675,000	91,900	13.3	28.0	1,879,000	93,300
103	18	25.3	4,407,500	120,500	13	26,9	2,093,000	76,200
104	17	24.3	4,920,000	190,000	13.3	24.6	4,025,000	113,900
106	17	21.3	3,667,500	141,000	13	22.2	3,104,000	98,300
107	17	19.6	2,082,000	91,200	13	20.5	1,180,000	19,300
Ave.	16.8	23.5	4,024,500	131,300	13.3	25.1	2,777,000	79,900

TABLE IV. COOK'S NO. II MEDIUM

	October 17				October 28			
Plot	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds
101	6.25	28.6	795,000	103,000	9.0	29.6	5,865,000	46,700
102	6.75	28.6	591,600	32,150	9.0	32.65	2,217,500	98,750
103	7.0	31.0	862,500	65,050	9.0	29.95	2,645,000	46,900
104	6.5	35.5	3,074,000	65,400	9.75	29.3	5,060,000	66,950
106	5.75	23.85	865,000	86,400	9.75	26.05	3,270,000	83,400
107	6.0	22.45	737,000	128,800	9.75	23.6	2,327,500	78,450
Ave.	6.37	28.33	1,154,200	80 130	9.37	28.52	3,564,200	70,190
	November 4				November 11			
101	7.5	29.45	4,676,500	81,300	5.5	32.05	3,561,000	78,000
102	8.0	31.4	3,200,000	20,850	5.0	32.35	1,953,500	30,450
103	8.5	31.85	7,737,500	104,450	5.25	31.0	2,207,500	47,150
104	9.25	29.3	5,774,000	64,900	5.0	29.2	4,160,000	33,600
106	9.25	24.3	3,324,500	82,050	5.75	26.7	4,245,500	96,250
107	9.5	22.6	2,012,200	59,700	5.25	25.25	540,500	53,000
Ave.	8.7	28.15	4,464,100	68,870	5.3	29.42	2,778,000	56,410
	November 20				November 28			
101	2.75	30.05	3,050,000	83,200	3.25	30.55	4,069,000	60,050
102	2.5	31.7	1,952,500	109,500	3.25	29.8	1,262,500	41,000
103	2.5	30.45	1,861,500	91,550	3.0	27.8	2,386,000	64,400
104	2.5	29.3	4,330,000	59,550	3.25	26.15	4,364,500	49,700
106	1.0	24.25	4,149,500	62,400	3.0	24.2	2,704,000	65,650
107	1.0	27.0	1,303,000	83,800	2.0	23.5	1,860,500	60,100
Ave.	2.4	28.8	2,774,400	81,660	3.12	27.0	2,774,400	56,820
	December 11				December 18			
101	1.5	30.35	4,600,000	52,400	−1.5	30.75	5,460,000	147,750
102	1.25	32.0	4,236 500	60,800	−2.75	31.25	2,761,500	38,050
103	1.0	30.3	2,437,500	50,650	−3	28.55	2,610,000	43,600
104	.87	25.65	3,740,000	123,600	−3	29.45	3,531,000	72,500
106	1.37	24.35	3,638,500	138,750	−4	26.5	1,966,500	109,600
107	.5	24.0	2,020,500	44,400	−3.75	22.9	1,043,500	19,250
Ave.	1.08	27.77	3,445 500	78,430	−3.0	28.23	2,896,400	71,790

TABLE IV. COOK'S NO. II MEDIUM (Continued)

Plot	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds
	December 27				January 6			
101	–1.25	35.55	3,384,000	62,350	–2.5	40.05	1,436,000	123,600
102	–2	32.45	1,861,500	80,750	–2.75	44.3	609,500	37,500
103	–2.25	35.75	960,500	12,300	–2.75	41.65	1,110,500	30,500
104	–2.5	36.5	1,709,500	21,950	–2.5	38.25	4,495,000	138,150
106	–2.75	42.2	2,350,000	36,650	–2.0	26.8	3,365,000	70,800
107	–3.0	32.2	945,500	43,600	–2.5	25.1	1,893,000	32,800
Ave.	–2.3	35.92	1,868,500	42,930	–2.5	36.02	2,151,500	72,220
	January 16				January 29			
101	–5	37.2	2,698,500	96,350	–2.13	52.95	2,852,000	44,100
102	–7	41.05	2,022,200	117,250	–2.25	42.1	2,682,000	47,050
103	–6.75	39.0	1,881,500	152,250	–2.75	37.0	2,732,000	68,650
104	–6.75	36.95	2,830,000	136,900	–2.0	42.9	2,711,000	65,350
106	–7.13	36.0	2,676,500	102,950	–2.0	33.0	2,202,000	75,100
107	–7.13	32.8	1,954,000	87,000	–2.13	35.55	519,500	27,100
Ave.	–6.63	37.3	2,343,800	115,450	–2.5	40.6	2,286,400	54,560
	February 12				February 22			
101	–5.5	32.25	6,890,000	104,500	–3.5	37.15	1,077,500	115,050
102	–6	49.45	4,070,000	59,100	–4.5	46.05	775,000	23,250
103	–6	38.9	2,405,000	131,650	–5	35.05	712,500	81,600
104	–6	36.5	5,742,500	65,050	–3.75	41.35	3,782,000	145,000
106	–6	32.2	4,227,500	92,450	–4.75	27.25	2,973,500	117,350
107	–6.25	28.05	1,031,000	38,700	–4.25	34.55	457,100	261,100
Ave.	–6.0	37.06	4,061,000	81,910	–4.3	36.9	1,629,600	123,890
	March 5				March 24			
101	–4	34.4	4,097,500	99,200	1	38.2	1,574,500	105,000
102	–6.75	41.6	3,137,500	70,100	1.8	42.9	1,325,000	28,700
103	–6	28.85	2,280,500	54,050	2.5	37.1	1,432,500	90,000
104	–6.75	35.25	3,952,500	67,850	2.0	35.4	1,980,000	69,000
106	–5	29.8	2,960,000	43,150	2.5	31.5	1,789,000	78,500
107	–6.0	28.85	1,815,000	21,150	2.0	27.9	932,500	41,500
Ave.	–5.7	33.12	3,040,500	59,250	2	35.5	1,505,600	69,000
	April 12				May 3			
101	4.5	34.1	3,455,000	128,000	7.5	39.4	7,120,000	115,500
102	5	30.8	3,715,000	116,500	7.0	39.0	3,430,000	50,000
103	6	31.3	4,345,000	51,400	7.8	34.2	3,820,000	66,200
104	6	30	4,305,000	69,000	7.3	33.4	4,555,000	131,500
106	5	26	3,330,000	135,200	6.8	28.9	4,610,000	72,300
107	5	23.1	2,017,500	57,000	6.5	24.9	1,845,000	41,200
Ave.	5.2	29.2	3,561,000	93,000	7	33.3	4,249,000	79,400
	May 29				June 19			
101	12.5	24.2	1,189,500	106,500	16.5	20.9	5,280,000	104,000
102	16	27.5	1,687,500	58,800	18.3	27.5	6,220,000	100,500
103	15.8	26.1	1,850,000	45,700	19	25.8	7,390,000	25,000
104	16	25	3,274,000	95,000	18.8	25.6	7,410,000	66,700
106	16	23.1	2,809,000	118,500	19.3	23.5	4,465,500	86,200
107	15	20.7	1,161,500	56,500	19	21.1	2,910,000	27,900
Ave.	15	24.4	1.996,000	80,000	18.5	23.9	5,612,600	68,300
	June 30				July 18			
101	18.8	19.7	3,685,000	113,700	20.8	22.1	....	61,000
102	22	25.4	2,667,500	123,500	25	25.9	....	69,400
103	22.4	25.5	2,790,000	53,800	25.5	26.5	....	149,300
104	22.4	24.5	3,880,000	82,200	25	35.6	....	120,500
106	22	22.1	3,400,000	96,500	27	22.9	....	111,000
107	22.3	18.9	2,225,000	66.500	26	20.9	....	90.300
Ave.	21.6	22.6	3,108,000	89,400	24.9	23.9	....	100,200
	August 6				September 3			
101	21	24.4	5,950,000	171,400	18.3	25.4	6,210,000	70,700
102	22	24.9	3,676 500	90 000	21.0	26 6	4,450,000	100,200
103	22.5	24.1	3,531,000	56,700	21.0	25.2	4,745,000	45,200
104	22.5	23.5	5,545,000	96,700	20.8	24	6,945,000	81,900
106	22	21.8	4 535,000	56,300	21	21.1	5,875,000	76,300
107	22.8	19.3	2,777,500	95,500	21	18.5	3,124,000	47,300
Ave.	22	23	4,336,000	94.400	20.5	23.4	5.225,000	70.300

TABLE IV. COOK'S NO II MEDIUM (5)

	September 29				November 2			
Plot	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds	Soil Temp. O°C.	H_2O pct.	Bacteria	Molds
101	15	24.4	5,360,000	157,500	14.5	28.7	5.167,500	52,900
102	18	26.1	4,865,000	126,000	13.3	28.0	4,937,500	67,100
103	18	25.3	4,725,000	90,400	13	26.9	5,310,000	70,800
104	17	24.3	5,200,000	82,000	13.3	24.6	7,205,000	99.700
106	17	21.3	5,620,000	76,200	13	22.2	5,960,000	85,800
107	17	19.6	2,991,500	83,500	13	20.5	3,223,000	55,500
Ave.	16.8	23.5	4,793,600	102,600	13.3	25.1	5,300,500	71,900

THE RELATION BETWEEN BACTERIA AND MOLDS

Comparing the mold content with the bacterial numbers, it is evident that there was no relation between the development of these two groups of organisms. In general, the bacteria decreased with the lowering of the temperature until December 27, when the ground was well frozen, the moisture varying only slightly during that period. After that date an increase in moisture occurred which was accompanied by a decrease in bacteria. On January 16, the first very low temperature was recorded and after that date the number of bacteria increased as the temperature dropped. This suggests that undoubtedly a change in the soil flora was taking place. The competing organisms were probably eliminated by the low temperature, as Conn suggests, and thus an abnormal increase was permitted of those organisms which are able to grow at these low temperatures. This, however, does not explain the increase in numbers of bacteria with a lowering of the temperatures below zero. His suggestion as to the crumbling action of frost on masses of bacteria does not offer an explanation, for this action would be quite constant in frozen soils, regardless of temperature.

Neither can it be explained by the capillary action in the soil, because at this period the soil was frozen several feet below the point where the sample was taken and any capillary moisture would be congealed on reaching this frozen soil and further movement would be prevented. The retarding effect of freezing on the development of protozoa would also be constant in all frozen soils and hence that effect would not be of significance here. Only one explanation remains, and that is that variations take place in the concentration of salts in the film of hygroscopic moisture, which according to the theory of Brown and Smith (4) is not frozen until the temperature goes very much below zero. In this work the temperature was not low enough to warrant the belief that the hygroscopic film was congealed.

After March 5, the soil thawed and was very muddy on March 24, which probably accounts for the low bacterial count on that date. The increase in temperature continued until it reached its

highest point on August 16, but the number of bacteria on this plot (101) increased to a maxium on April 12 after which a decrease occurred toward fall, altho the moisture content remained almost constant and the temperature increased. On the other plots in the series tested which were kept fallow, the maximum count was not obtained until June 19. Thus it appears that seasonal variations in cropped and uncropped soils are essentially different. There evidently must be some other condition than moisture and temperature which exerts a controlling influence on the bacteria in plot 101 after April 12. Just what this might be would, of course, be mere speculation. It is probably microorganic in nature and may perhaps be protozoa. Chemical conditions might account for the results, at least in part.

VARIATION IN BACTERIA DURING SEASONS

These results show that bacteria in the soil, at least those species which develop on the three media used here, may increase or decrease with the temperature and moisture conditions during the fall season or at least during the time when the soil is not frozen. When the soil is frozen, but the temperature does not go so low that the hygroscopic moisture freezes, the bacteria may increase regardless of moisture or temperature. Similarly, during the summer the bacteria may be influenced by some other factor and increase or decrease, regardless of moisture and temperature. During the growing season, however, extremes both in moisture and temperature affect bacterial development. Apparently the effect on bacteria is no indication of any influence on molds and the latter organisms develop under the control either of their own life cycle or of some condition as yet unrecognized.

The development of both the molds and bacteria was somewhat different on the three media but in the above discussion the general tendencies on all three media have been considered. In the case of molds, there is so little difference in the growth that no general comparison can be made. The character of the growth is quite different, however, especially on Cook's No. II, from that on the other two media. The albumen agar appears to give somewhat lower results on the average than the other media. It is apparent that accurate quantitative work with molds will require the preparation of special media.

In the case of the bacteria the highest counts were obtained on the albumen agar in practically all cases. The modified synthetic agar medium gave the next largest count, while the Cook's No. II was the lowest. This was expected, as the latter medium is especially designed for mold growth.

THE NUMBER OF MOLDS IN SOILS

The actual number of molds in the soil as shown by these results with the three media ranged from 12,000 to 261,000. The average counts obtained on the three media from the samples from all the plots ranged, however, from 42,000 to 131,000. High individual counts may often be secured as pointed out above and hence the average counts probably represent more nearly the numbers of molds occurring in these soils under normal conditions. The previous investigations of numbers of fungi in soils were reviewed by Waksman (18) and need not be cited here. Waksman himself found 6.2 to 7.1 pct. of the total microorganic flora of soil to be fungi which amounted to 400,000 to 1,100,000 per g. of soil at a depth of 1 inch and 7.9 to 11.7 pct. at a depth of four inches. In cultivated non-acid soils the ratio of bacteria to fungi was 10 to 1, while in forest soil it was 5 or 6 to 1. Some of the earlier investigators found a ratio of 10 to 1 in some soils while the ratio widened or narrowed according to the soil conditions and treatment.

It is hardly worth while to discuss the ratio secured in this work except to say that it was much wider than found by previous workers. On the average, the number of molds developing on the media used was much smaller in relation to the bacteria than found by others. This may be due to the media used, altho the ratio is not very different on the three widely different media. The media may be better adapted to the growth of bacteria permitting of larger counts, or the mold growth may more nearly represent the active mold forms in the soil because of the method of study and particularly the time of incubation, altho the latter varied with the different media. The ratio secured in this work for general average counts was about 40 or 50 to 1.

In spite of this comp ratively small number of molds in relation to bacteria it does not seem advisable to conclude that they are unimportant. It is quite possible that a small number of molds may be much more important than a very large number of bacteria, if the molds are concerned in some process particularly important from the soil fertility standpoint while the bacteria are not active or less active in such a process. Until more is known about the species of molds and their action in the soil it is not wise to conclude *even* that they are *less* important than bacteria and certainly not that they have no influence on soil fertility. Altho direct microscopic examination of soils may not show the presence of mold mycelia, a rather large number may be present and their action may be very important. Neither should too much emphasis be placed upon mold occurrence and activities in the soil and this work serves to show that the num-

ber of molds may be very much smaller in some soils, altho they are rich in organic matter, than has previously been found.

The following charts, II, III, IV, V, VI, need not be discussed at length, as the general conclusions regarding mold growth thruout the season are verified by the results secured on differently treated soils. Some differences are apparent and these will be noted briefly, but in general the conclusions reached with plot 101 are confirmed.

The results secured from the soil on plot 102, which receives 2.8 tons peat annually, are shown in chart II. This plot was characterized by a uniformly low bacterial content which was probably due to the peat application. The moisture content remained practically constant until after December 27, when it increased but this increase was accompanied by a low bacterial count. On February 12, the moisture was the highest and the bacterial count was also the highest, but the temperature on that date was very low and no definite conclusions regarding the effects of moisture and temperature are possible. It is of interest to note that not until after the temperature had reached a minimum was there an increase in bacteria on all media. The results in general confirm the previous ones. There again an increase in bacteria occurred during the continuance of the freezing period. As in the other plot, the number of molds seems to be quite constant regardless of moisture and temperature. It should be noted here that the low count of bacteria on the synthetic agar on January 16 was probably due to the overgrowing with molds, as a high mold content was found on that date.

The results from plot 103, which receives 8 tons of manure every four years (1909, 1913), are shown in chart III. A very high bacterial count was found on November 4 and there was also an increase in mold colonies on that date. This is practically the only variation in the counts on this plot from those secured on the other plots. It seems probable that there was an abnormal amount of organic matter in the particular spot from which the sample was drawn. The low count on the albumen agar on that date was undoubtedly occasioned by a crowding out of the bacterial colonies by molds.

The results in general confirm the previous conclusions. After the minimum temperature was reached in the frozen soil, a rise in temperature suppressed the bacterial count (see January 29) and a decrease in temperature caused a simultaneous increase in the bacterial count (see February 12) regardless of the moisture content. This is just what was noted in the results from the other plots. Here again the number of molds was not materially affected by either temperature or moisture, but

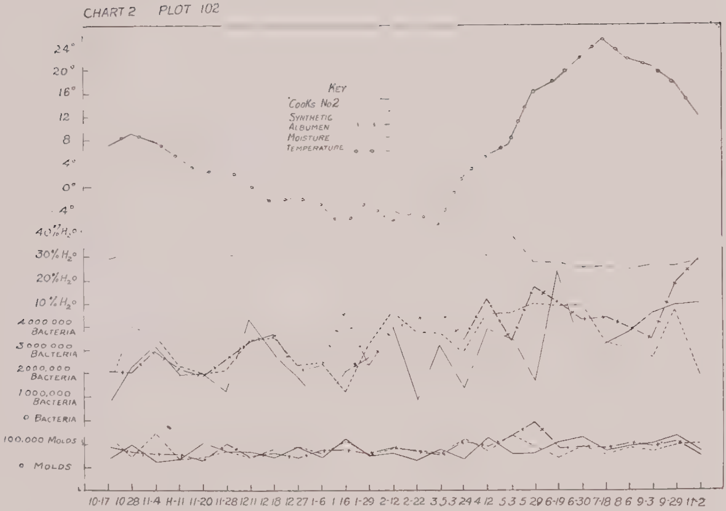

Chart II. Bacteria and molds in soil from peat plot

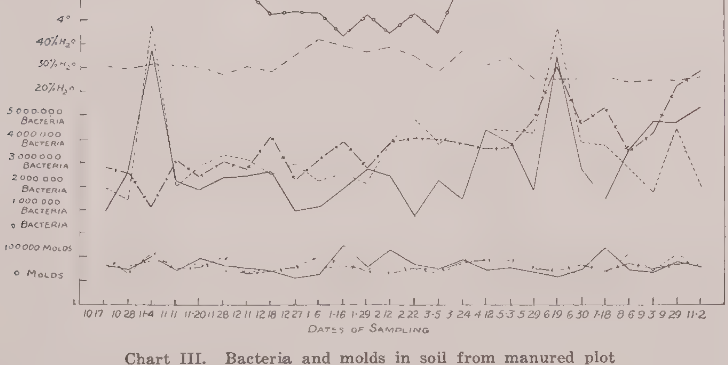

Chart III. Bacteria and molds in soil from manured plot

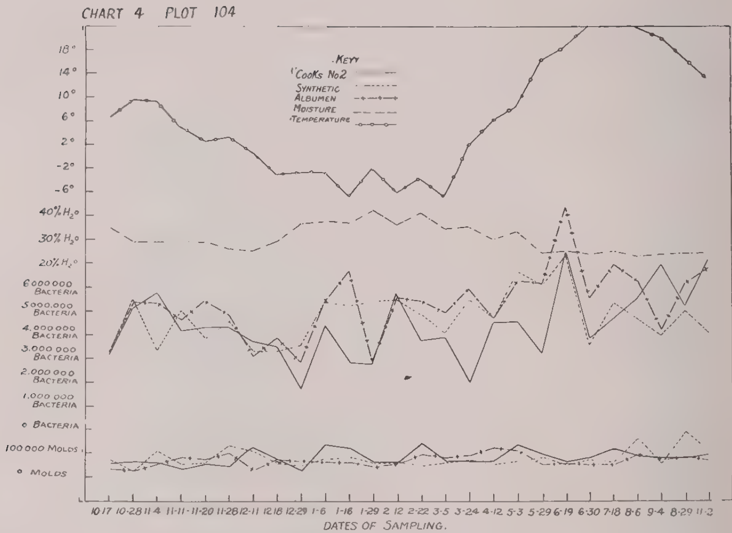

Chart IV. Bacteria and molds in soil from clover-treated plot

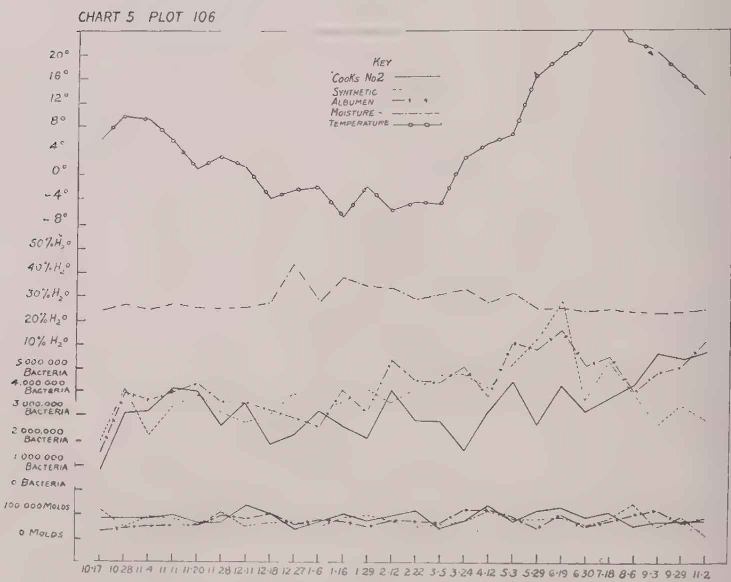

Chart V. Bacteria and molds in soil from timothy-treated plot

fluctuated somewhat regularly thruout the season, dependent evidently upon some other factor or condition peculiar to those organisms.

In chart No. IV there are shown the results obtained on plot 104 which receives 8 tons of clover hay every four years (1909, 1913). It is of particular interest to note how the temperature curve intersects those representing the bacterial count during the frozen season as one decends the other ascends and vice versa. As in the previous cases, a retarding effect on bacterial numbers was brought about by a decrease in soil temperature until the soil was frozen. After that, however, the numbers increased with depressed temperatures and decreased with increases in temperature regardless of the moisture. Again as noted in the other plots, the number of molds was not influenced by moisture or temperature and did not seem to bear any relation to bacterial numbers. The fluctuations in mold numbers was likewise very much the same as observed in the other tests.

Chart No. V gives the results secured for plot 106 which receives 2 tons of timothy annually. The chief point to be noted here is that there was no great retarding effect on numbers of becteria as the temperature dropped in the fall. This is probably due to the plowing under of the application of timothy which takes place at that time in the year. In all other respects the results confirm the observations previously noted. The number of bacteria rose and fell with lower and higher soil temperatures after the soil was frozen and showed no relation to the moisture conditions. The mold growth again was not influenced by moisture or temperature but flucutated thru the season as in the other cases.

The results obtained on plot 107, which is a check plot, are shown in chart VI. In this case there was no great increase in bacterial numbers during the frozen period but a uniformly high count was found at the period of lowest temperature. The failure of the plot to show a decided inrease in numbers of bacteria while the soil was frozen was in direct contrast to the results on the other plots. This being a check plot, higher in topography and low in organic matter, it is possible that the concentration of the soil water would be sufficiently weak to permit of the freezing of much of the hygroscopic moisture. This is in accord with the theory of Brown and Smith (4) and might explain the variation in these results. The large count of molds on February 22 is probably due to fructification of a mold in the sample. Other than this the numbers of molds seem as usual to be unaffected either by moisture or temperature.

THE EFFECTS OF SOIL TREATMENT

Comparing the results secured on all the plots, some interesting facts are brought out. The greatest bacterial count was ob-

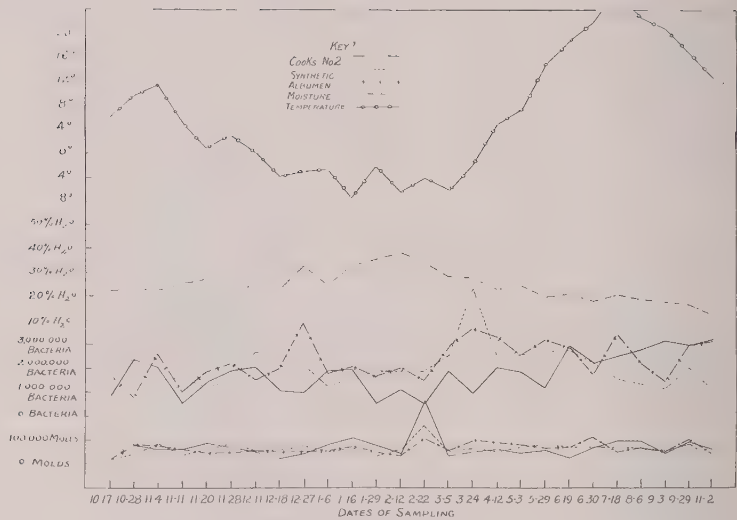

Chart VI. Bacteria and molds in soil from check plot

tained on plot 104, which received 8 tons of clover hay every four years. Altho nearly four years had elapsed since the last application, a marked influence could be seen. Plot 101, in continuous timothy meadow, ranked second. Slightly less than this was plot 106, which receives 2 tons of timothy annually. Evidently the treatment of these two plots is of less significance than the topography for it would be expected that the cultivated plot receiving the treatment with timothy would be much higher in bacteria than the timothy meadow plot. In topography, however, plot 101 is lower than plot 106 and therefore it is not only naturally richer in organic matter, but continues to be enriched constantly. Some other factor than plant food content or organic matter present might account for the high results on plot 101 but no investigation of this point was carried out.

In plot 103, which receives 8 tons of manure once every four years, it was quite unexpected to find the numbers very low in comparison with plot 104, the highest in bacteria, where clover was applied once in four years. The difference in topography is not sufficiently great to warrant the variation in numbers which occurs, nor would it be expected that clover would increase the bacteria more than manure. Of course, it is possible that the effect of the clover persists for a longer period than that of the manure. However, it seems that some factor not studied had more influence on the bacteria in these plots than the treatments to which they were subjected.

The low count on plot 102 is probably due mainly to the peat

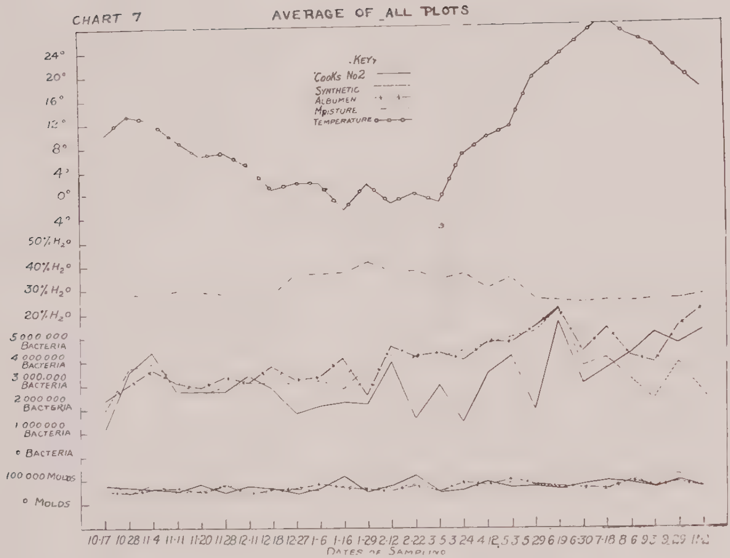

Chart VII. Average bacteria and molds from all plots

applied, altho the topography of the plot is such that water tends to pond in the center and the physical conditions may be the controlling factor in bacterial growth. The check plot (107) is the lowest in bacteria of all the plots and this might lead to some conclusion regarding treatment, but the topography of that plot in undoubtedly an important factor in bacterial development. The plot is higher than the others and naturally poorer in plant food and organic matter. In general, the effects of soil treatment in these plots on the bacteria is apparently subordinate to other factors and while some effects are noted, general conclusions should not be drawn. Especially since the results are so unusual it does not seem wise to make any broad interpretations of the data.

The soil treatments did not seem to affect the growth of molds, materially. The smallest numbers were found in plots 103 and 107 just as in the case of bacteria, and the highest count was likewise in plot 101. The difference in the case of the molds were not very great and it must be concluded that some other factors than soil treatment were of more importance in mold growth.

THE AVERAGE COUNTS ON ALL PLOTS

The average counts on all the plots for the three media are shown in chart VII. Altho the depressing effect of decreasing

temperature in the fall does not appear as clearly as the other charts, the depression being obscured by the results on plots 106 and 107, the chart shows clearly a great increase in numbers after January 16. The increase was reduced by a rise in soil temperatures and greatly increased by a drop in temperature, regardless of the moisture. Furthermore, the increase in bacterial numbers observed was greater than that found in unfrozen soils. There was an increase in bacteria again following the decrease and this increase occurred at the time of the thawing of the soil. The highest count was obtained on June 19, after which a decrease occurred, altho the temperature continued to increase and the moisture remained constant. The two maximum counts of the year were obtained on February 12 and on June 19, with periods of minimum counts intervening. These results confirm the earlier work of Conn and Brown and Smith already referred to, and show that bacteria are active in frozen soils and also that there may exist groups of bacteria especially adapted to grow under winter or summer conditions. There may be such groups as "winter" and "summer" bacteria. No conclusions can be drawn relative to the effect of moisture and temperature. In the frozen soil, neither seems to have any effect while in unfrozen soils, temperature sometimes seems to control (as in the fall) but at other times (as in the summer and second fall) neither temperature nor moisture have any apparent influence.

The number of molds, as indicated in all the charts, does not seem to be influenced at all by seasonal conditions. The number rises and falls irrespective of moisture and temperature and is apparently dependent on some factor not studied. Neither is there any relation between the bacterial growth and the mold growth. They are apparently little related to each other and not only proceed independently but are differently affected by the influence of various factors.

SUMMARY

This study of bacteria and molds in the soils of six differently treated plots thruout one year permits of the following conclusions:

(1) The bacteria decreased in the late fall with a drop in temperature until the soil became frozen.

(2) In frozen soil, the number of bacteria rose with decreased temperatures and fell with higher temperatures, regardless of the moisture content.

(3) Upon the thawing of the soil, the number of bacteria decreased. With increasing temperature, however, an increase in bacteria occurred which reached a maximum on June 19 in all the cultivated plots and on April 12 in the continuous timothy plot.

(4) There were two maximum counts during the year—on February 12 and June 19, with intervening minimum counts.

(5) During the summer and early fall, the bacteria did not develop parallel with either moisture or temperature.

(6) During much of the year other factors than moisture and temperature or general seasonal conditions seem to control bacterial development.

(7) The treatment of the plots led to some unexpected effects on the bacteria. Applications of peat depressed the bacteria. Manure and clover increased the number of bacteria. The continuous timothy plot showed the largest number of bacteria present but this may have been due in part at least to the topography of the plot.

(8) The number of molds present in the soils fluctuated from one sampling to the next but was apparently unaffected by moisture, temperature or soil treatment. Some factor as yet uninvestigated probably accounts for this fluctuation.

(9) The actual number of molds present in these soils was much smaller than shown by previous investigations, ranging from 42,000 to 131,000 on the average for all the plots. The number generally amounted to one-fortieth to one-fiftieth of the bacteria present, depending upon the medium used. There was apparently no relation between the bacteria and the molds present in the soil.

(10) The small number of molds in soil compared with bacteria may not necessarily mean that they are less important and certainly will not prove that they are unimportant.

(11) With the three media used the albumen agar gave the highest count of bacteria, the modified synthetic agar was second and Cook's No. II third. In the case of molds, the albumen agar gave the lowest counts while the other two were about the same.

(12) Active mold growth has been shown in normally cultivated soils by the development of mycelia from small portions of soil when inoculated into agar plates. Development from spores is very much slower than from active mycelial forms. The presence of mold spores in the soil is believed to be of importance from the fact that their occurrence presupposes the *previous* presence of active forms and hence the *future* development of active mycelia may be expected if the soil conditions become satisfactory. There is nothing yet to disprove the idea that molds go thru thei regular life-cycle in the soil.

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